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ORIGINAL PAPER

Nucleolar marker for living cells

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Abstract In the recent molecular and cell biological research, there is an increasing need for labeling of subcellular structures in living cells. Here, we present the use of a fluorescently labeled cell penetrating peptide for fast labeling of nucleoli in living cells of different species and origin. We show that the short peptide with ten amino acids was able to cross cellular membranes and reach the nucleolar target sites, thereby marking this subnuclear structure in living cells. The treatment of cells with actinomycin D and labeling of B23 protein and fibrillarin provided evidence for a localization to the granular component of the nucleolus. The fluorescently conjugated nucleolar marker could be used in combination with different fluorophores like fluorescent proteins or DNA dyes, and nucleolar labeling was also preserved during fixation and staining of the cells. Furthermore, we observed a high stability of the label in long-term studies over 24 h as well as no effect on the cellular viability and proliferation and on rDNA transcription. The transducible nucleolar marker is therefore a valuable molecular tool for cell biology that allows a fast and easy labeling of this structure in living cells.

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Abbreviations

AMD	Actinomycin D
DRAQ5	Deep red fluorescing anthraquinone Nr. 5
DMEM	Dulbecco's modified eagle medium
FACS	Fluorescence activated cell sorting
FCS	Fetal calf serum
FITC	Fluorescein isothiocyanate
FU	Fluorouridine
GFP	Green fluorescent protein
HEPES	*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid
HIV	Human immunodeficiency virus
mRFP	Monomeric red fluorescent protein
PI	Propidium iodide
PCNA	Proliferating cell nuclear antigen
TAT	Transactivator of transcription

Introduction

The nucleolus is the most prominent substructure in the cell nucleus and it was first described in 1836 by Gabriel Gustav Valentin (Franke 1988). It took more than one century to establish its role in ribosome biogenesis (Brown and Gurdon 1964; Perry 1962). The nucleolus is a very dynamic structure and it forms around the rDNA loci (Andersen et al. 2005). The inner nucleolar structure is organized into fibrillar centers (FC, where rDNA is located and at the periphery of which transcription takes place) surrounded by the dense fibrillar component (DFC, into which nascent

transcripts migrate) and all around the granular component (GC), which is filled with ribosomal precursors (Cheutin et al. 2002; Derenzini et al. 2006; Scheer and Hock 1999). Long ago, it has been noted that the nucleolus size reflects the cellular activity, proliferation and differentiation (Hernandez-Verdun 2006). Furthermore, the nucleolus bears the hallmark of other subnuclear compartments, in that is not delimited by membranes, as is the case for cytoplasmic organelles.

Owing to its higher density and refractility in comparison to the surrounding nucleoplasm, the nucleolus can be visualized with phase contrast or differential interference contrast microscopy. However, the three-dimensional volume and localization within the nucleus as well as its exact borders are hard to identify by contrast microscopy alone and require a fluorescence label (Lam et al. 2005). In addition, such a marker would facilitate high throughput analysis to sort nuclear proteins according to their intranuclear localization. Finally, the development of new high-resolution optical techniques allows for the first time optical analysis down to the nanometer scale but require fluorescent labels. This gain in information could help to elucidate the functional nuclear and genome organization in relation to the nucleolus in the three-dimensional nuclear structure. Specific antibodies to nucleolar proteins or the transfection of cells with plasmids encoding for fluorescent fusions of nucleolar proteins allow the visualization of the nucleolus by fluorescence microscopy. However, these methods require either fixation of the sample and immunostaining in the case of antibodies or transfection of cells and expression of the fluorescence marker in the case of fluorescent proteins. The latter strategy has the advantage of allowing live-cell microscopy but is first time consuming and second restricted to transfectable cells, which is not the case of most primary cells. Furthermore the analysis of drug effects on the nucleolar organization and function could be simplified by using a fast nucleolar label for living cells. A membrane-permeable fluorescent label for the nucleoli, which is fast and easy to apply on living cells, would therefore be a very useful molecular tool for diagnostics and cell biological research.

The ability of certain peptides and proteins with concentrated basic charges to cross cellular membranes was earlier discovered in 1988 for the HIV TAT protein (Frankel and Pabo 1988; Green and Loewenstein 1988), and the peptide domain responsible for membrane transduction was mapped a decade later (Vives et al. 1997). Further comparison of native and artificial membrane transducing peptides indicated that arginines are superior to lysines in transduction potential (Mitchell et al. 2000; Thoren et al. 2003). Independent studies found that peptide sequences present in several viral and cellular proteins containing a stretch of 6–10 basic amino acids could serve as a targeting sequence for the nucleus and the nucleolus (Dang and Lee 1989; Hatanaka 1990). In this work we reasoned that if both properties, membrane transduction ability and nucleolar targeting signal, could be combined in the same peptide made of a series of basic amino acids we could exploit these independent biological functions to directly label the nucleolus in living cells.

Materials and methods

Peptides and plasmids

Amino-terminal fluorescein labeled deca-arginine peptide (FITC-R_{10}) was synthesized with D-amino acids by Peptide Specialty Laboratories (Heidelberg, Germany), dissolved in ddH_2O and, for application on living cells, further diluted in growth medium.

The mRFP-PCNA expression construct was described before (Sporbert et al. 2005).

Cell culture and transfection

Human HeLa cells as well as Flp 3T3 mouse fibroblast cells were cultivated in DMEM with 10% FCS + 5 mM L-glutamine and 5 μg/ml Gentamycine. C2C12 mouse myoblasts were grown in DMEM with 20% FCS and the same additives. Transfection of C2C12 cells with mRFP-PCNA was carried out by $CaPO_4$ precipitation method as described (Cardoso et al. 1997). For live cell microscopy the cells were plated onto 4- or 8-well Lab-Tek coverglass chambers (NalgeNunc) or 8-well Ibidi chambers (Ibidi). Adult ventricular cardiomyocytes were enzymatically isolated by retrograde perfusion from excised adult male Wistar rat hearts at a constant flow of 6 ml per min with a Ca^{2+}-free HEPES solution containing collagenase (Worthington type II, 60 IU per ml). The freshly dissociated cells were kept at physiological solution with 0.3 mM Ca^{2+} and 0.5% bovine serum albumin at room temperature (Alvarez et al. 2004). DRAQ5 (Biostatus Ltd) staining of DNA in living cells was as described (Martin et al. 2005).

Transduction assays

All peptide transduction experiments were performed with living cells plated on 4- or 8-well coverglass chambers. Before observation, the medium was removed and growth medium with the different peptide concentrations was directly applied to the cells. To remove the background fluorescence of extracellular peptides a

washing step with PBS was included after 30 min of incubation followed by incubation in growth medium without peptide. For the concentration dependent uptake (Fig. 3a) the cells were incubated with the peptides for 1 h before washing with PBS while for the continuous time series to study the uptake dynamics (Fig. 3b) no washing step was performed. The uptake of the marker peptide was studied under a fluorescence microscope for 1 h starting with the peptide application. After observation and imaging of the nucleolar label the cells were returned to the incubator and further image collection was performed 24 h later. Transduction efficiencies were determined in two independent experiments by counting the number of transduced cells with nucleolar label directly on the fluorescence microscope in ten fields of view ($n = 130–180$ cells).

Cell viability and proliferation assay

Cell viability and label stability after nucleolar marker application were monitored by the ability of the cells to undergo complete mitotic cell cycles and grow to confluency as well as monitoring the label intensity in the nucleoli 24 h after application.

The membrane integrity was ascertained by the trypan blue exclusion assay. Into each well of a 4-well LabTek chamber with 400 µl medium 2 µl trypan blue solution (Sigma) was added and mixed by pipetting up and down. The number of trypan blue positive cells was counted at 30 min and in separate samples at 24 h.

The impact of the nucleolar marker on cell viability and cell cycle progression was assayed by plating equal number of cells and 1 day later adding or not 10 µM FITC-R_{10} to the media for 1 h followed by a washing step in PBS and incubation in growth medium. The next day, cells were trypsinized, fixed with methanol for 20 min and stained with 50 µM propidium iodide (PI) (Sigma) for 1 h in the presence of 0.1 mg/ml RNase A (Sigma). DNA content was analyzed by flow cytometry (Becton Dickinson FACSVantage using 488 nm laser excitation and 675/20 nm bandpass filter for detection) as a measure of cell cycle progression (Plander et al. 2003). Five sets of 1×10^4 cells and five sets of 2×10^4 cells were analyzed, and all the data sets were used for the calculation of mean values of cells in

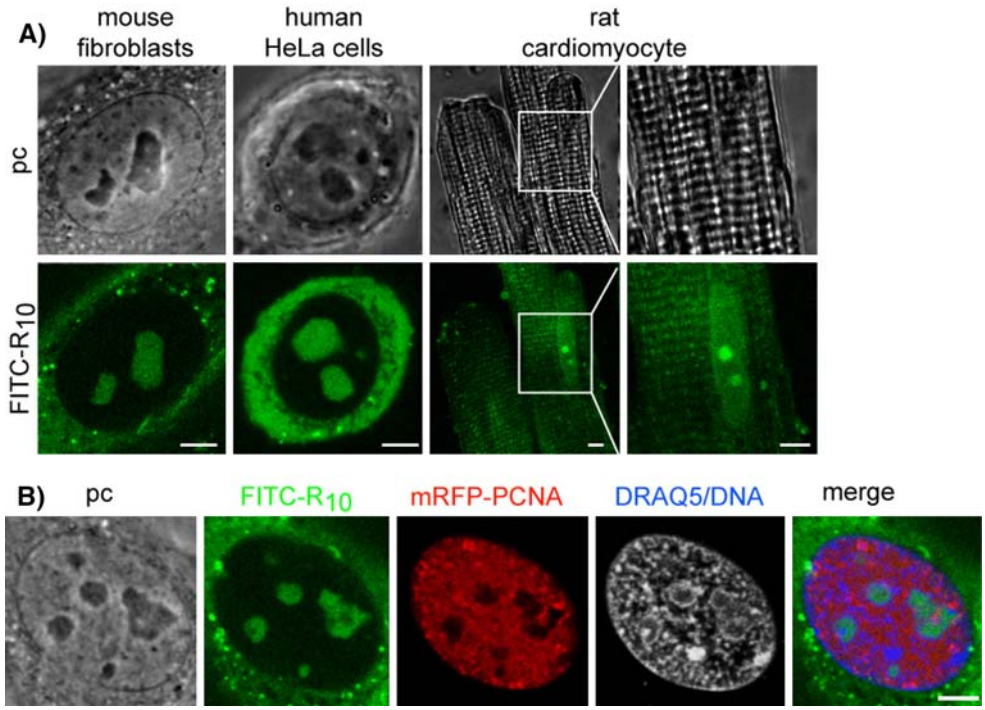

Fig. 1 Transducible nucleolar marker uptake into different cell types and in combination with other fluorophores **a** This figure shows the fluorescently labeled nucleolar marker taken up into cells of different species and origin, which are either from cultured cell lines (fibroblasts, mouse; HeLa, human) or primary cells that are terminally differentiated (adult cardiomyocyte, rat). The cells were incubated with a medium containing the transducible nucleolar marker for 30 min and then washed in PBS and supplied with the medium. In both cases the marker transduced fast into the cells where it accumulated in the nucleoli and thereby marked this subnuclear structure. **b** Displays a C2C12 mouse myoblast transfected with a plasmid coding for mRFP-PCNA (*red*) and with DNA stained by DRAQ5 (*blue*). The transducible nucleolar marker labeled with FITC (*green*) can be used in combination with fluorescent protein labels like mRFP as well as fluorescent dyes such as the live cell DNA stain DRAQ5. Scalebar: 5 µm

G1, S and G2/M phase for FITC-R_{10} labeled and control cells using ModFit 3.0 software.

In situ transcription assay and inhibition with actinomycin D

Cells were grown on glass coverslips and 1 day later incubated with FITC-R_{10} for 1 h or 24 h in a 12-well plate. Actinomycin D (AMD) treatment was performed at 0.04 mg/l for 2 h before adding FITC-R_{10} at 10 μM to the same growth medium or for 2 h after the incubation of cells with FITC-R_{10} for 1 h. For the labeling of nascent RNA cells were incubated with 1.5 mM FU in growth medium for 10 min and washed in PBS.

After the incubation schemes cells were fixed in 3.7% formaldehyde in PBS for 10 min and permeabilized with 0.25% (0.5% for AMD experiments) Triton X100 in PBS for 10–12 min. The samples were immunostained with anti-B23 mouse monoclonal antibody (clone FC82291; Sigma) followed by detection with donkey anti-mouse IgG antibody conjugated with TexasRed (Jackson) and #346 anti-fibrillarin human autoantibody (kind gift from P. Hemmerich, FLI Jena) followed by donkey anti-human IgG biotin SP (Lot: 47441 Jackson) and streptavidin-Cy5 (Amersham). Fluorouridine incorporated into RNA was detected with anti-BrdU rat monoclonal antibody (clone BU1/75; Harlan Sera Lab) followed by incubation with donkey anti-rat IgG antibody conjugated with Cy5 (Jackson). For the labeling of DNA cells were counterstained with TOPRO-3 at 1.3 μM final concentration in PBS (Molecular Probes) and mounted with Moviol.

Microscopy, image acquisition and analysis

Live cell microscopy was performed with a Zeiss LSM510Meta confocal setup mounted on an Axiovert 200 M inverted microscope using a 63× phase contrast plan-apochromat oil objective NA 1.4 heated to 37°C. For all acquisition settings the main beam splitter was HFT UV/488/543/633 and the parameters specific for each fluorochrome are listed below:

Fluorochrome	Ex (nm)	Em (nm)
FITC	488	BP500–530
mRFP, L-Rhodamine	543	BP565–615
DRAQ5, Cy5	633	LP650

Imaging of AMD treated cells was done at a Leica TCS SP5 confocal setup mounted on a Leica DMI 6000 CS inverted microscope using a 63× HCX plan-apochromat DIC oil objective NA 1.4. The image acquisition was done sequentially to minimize potential crosstalk between the fluorophores. The trypan blue exclusion was determined with brightfield illumination on the Zeiss LSM510 microscope setup. The uptake kinetics analysis (Fig. 3b) was performed by selecting ten nucleoli in ten individual cell nuclei in the image of the last time point and determining their mean fluorescence intensity (FI) at each time point. The fluorescence intensity data for all individual nucleoli were averaged and plotted against the timescale. Labeling of nascent RNA by FU was analyzed by selecting 60 nuclei from confocal images and determining the mean fluorescence intensity. Background correction was done by applying a threshold according to the mean fluorescence intensity in images from control cells without primary antibody (anti-BrdU). Image analysis was performed with Zeiss LSM Image examiner 3.2 (Zeiss) and Origin 7.5 software (Origin Lab Corp.).

Results and discussion

Our goal was to develop an easy to use live cell fluorescent marker for the nucleolus. Therefore, we tested whether nucleolar targeting ability could be combined with cell penetrating ability in one peptide. The latter would allow non-invasive application to all sorts of cells. We chose a peptide composed of 10 arginines, which had been shown to efficiently transduce into cells (Wender et al. 2000). To prevent proteolytic degradation the peptide was synthetized with D-amino acids. The fluorescent label (FITC) was added to the N-terminal end.

First, we tested the ability of this peptide (FITC-R_{10}) to be taken up by different cells and its intracellular localization. We selected different cell types (fibroblasts, epithelial, muscle) from different species (mouse, rat, human) and not only cell lines (Flp 3T3, HeLa) but also primary cultures (cardiomyocytes). FITC-R_{10} was diluted in growth medium and applied directly to the cells for 30 min. After the incubation time the cells were washed and analyzed by confocal microscopy. In all cells tested (Fig. 1a) the fluorescence label was found accumulated in the nucleoli identified by the phase contrast images (except in cardiomyocytes where the nucleoli cannot be detected due to the sarcomeric structures) and also diffusely distributed in the cytoplasm. The transfection of the cells with an S-phase marker (mRFP-PCNA; Sporbert et al. 2005 and simultaneous DNA labeling with DRAQ5 Martin et al. 2005) indicated that uptake of the peptide was not cell

cycle dependent. Indeed, cells in different stages of the S-phase as well as in G1/G2 exhibited nucleolar labeling and also mitotic cells had taken up the marker although the nucleus and the nucleoli are not present (Fig. 1b and data not shown). These data also indicate that combinations with other fluorochromes (e.g., live cell DNA staining with DRAQ5) and fluorescent proteins (e.g., mRFP) are possible. Since any other fluorescent label can be selected for the nucleolar marker peptide, this allows multiple combinations with other fluorochromes.

The FITC-R_{10} label colocalized with the dark structures in the phase contrast images, which were surrounded by dense chromatin detected by TOPRO-3 staining (Fig. 2a). To confirm the nucleolar localization of FITC-R_{10} we performed colocalization studies with nucleophosmin/B23 (Fig. 2b), a protein present in the granular component of the nucleolus and fibrillarin localized in the dense fibrillar component (reviewed in Olson and Dundr 2005). The FITC-R_{10} label colocalized with the B23 antibody signal at the periphery of the nucleoli and filled the nucleolar interior showing a decreased concentration in the dense fibrillar components labeled by fibrillarin (Fig. 2b). This demonstrated that the FITC-R_{10} peptide possesses intracellular nucleolar targeting ability and furthermore localizes to the granular component of the nucleolus.

To further test the exclusion of FITC-R_{10} from fibrillar components we treated cells with AMD. Exposure of cells to this transcription inhibitor leads to a separation of fibrillar and granular components into distinct caps (Reynolds et al. 1964; Chen et al. 1999). The AMD treatment of cells before or after (data not shown) labeling the nucleoli with FITC-R_{10} showed the persistent localization of FITC-R_{10} to the granular component remnant separated from the caps formed by the fibrillar components without FITC-R_{10} (Fig. 2b). In contrast to B23, the nucleolar marker did not redistribute throughout the nucleoplasm after AMD

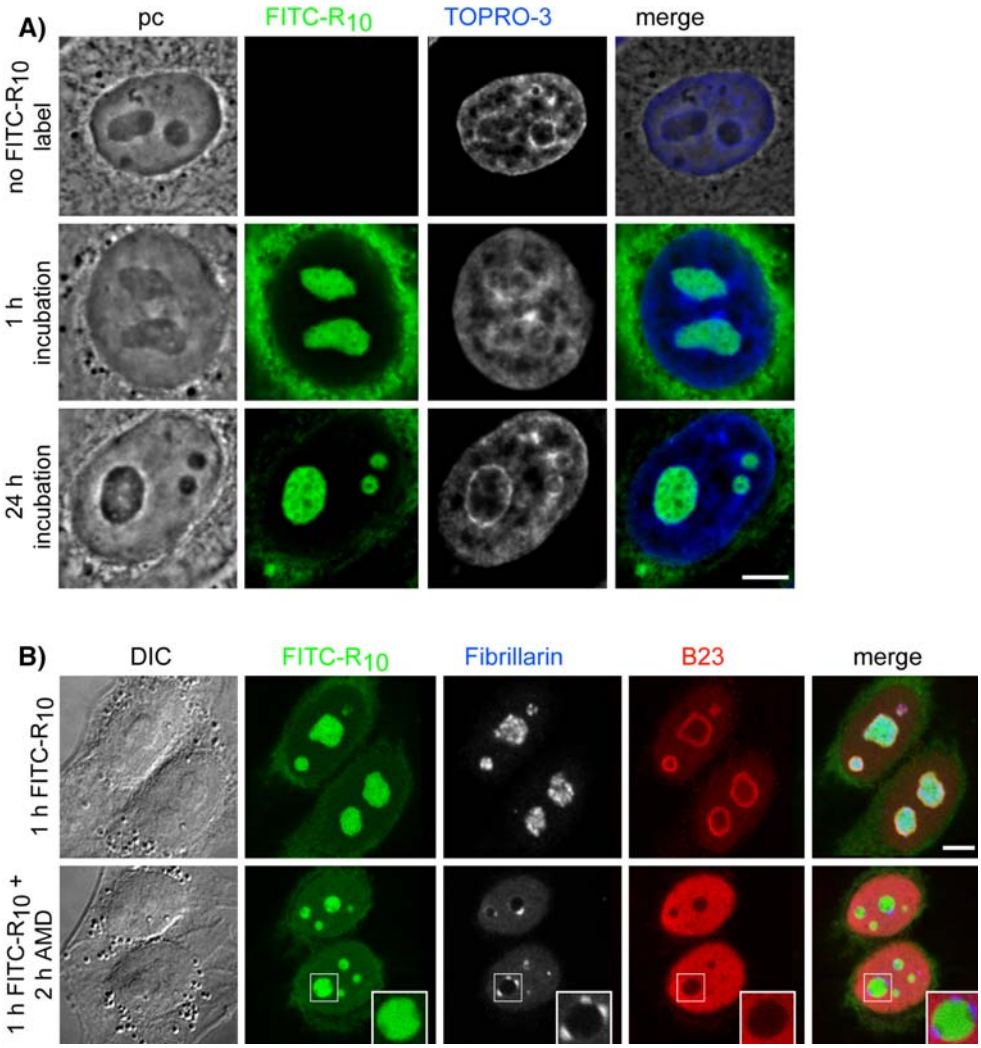

Fig. 2 Intracellular localization of the FITC-R_{10}. **a** HeLa cells incubated for 1 and 24 h with 10 μM FITC-R_{10} as well as unlabeled controls were fixed in 3.7% formaldehyde in PBS and stained with TO-PRO-3 to label the DNA. The FITC-R_{10} label colocalize with the nucleoli identified in the phase contrast during short- and long-term incubation of the cells. **b** The HeLa cells were immunolabeled for B23/nucleophosmin to highlight the granular component of the nucleolus and fibrillarin to label the dense fibrillar components. One sample was treated with actinomycin D (AMD), which leads to a stop in rDNA transcription and the formation of nucleolar caps containing the fibrillar components. The FITC-R_{10} label was still localized to the nucleolar interior unlike B23, which was redistributed to the nucleoplasm and excluded from the nucleoli. The nucleolar marker does not colocalize with the fibrillarin labeled caps in AMD treated cells. Scalebar: 5 μm

treatment indicating that it interacts with other molecules of the granular component. Thus, the FITC-R_{10} can also be used in cells where rDNA transcription was stopped to label the granular components of disassembled nucleoli.

Furthermore, these immunostaining experiments after live cell application of FITC-R_{10} demonstrated that fixation and further treatments could be performed without loosing the label or changing its localization (Fig. 2 and suppl. Fig. 1A). When FITC-R_{10} was applied to pre-fixed samples though, it did not stain the nucleolus (suppl. Fig. 1A).

The kinetics of uptake of the peptide into cells was then studied by time-lapse confocal microscopy. The time series analysis is shown in Fig. 3b and in the supplementary online movie. The images at the first and last timepoints display the accumulation of the marker over time inside the cells and on the cell membrane. The graph represents the fluorescence increase in the nucleoli of 10 different cells. The peptide was rapidly internalized and labeling of the nucleoli started around 20 min and reached the equilibrium at 40 min. Earlier nucleolar labeling could have been missed due to the strong extracellular fluorescence of non-internalized peptides.

In a separate set of experiments we performed a dose response uptake analysis. Different concentrations of the marker in the growth medium were applied for 1 h to HeLa cell cultures. A threshold minimal concentration for nucleolar labeling was found at 2.5 μM. Lower peptide concentration (1 μM) resulted in no uptake of the marker into the cells (Fig. 3a). A possible explanation for this finding is that a certain concentration of the basic CPP on the cell membrane needs to be reached to enable the membrane transduction process (Dietz and Bdeltahr 2004; Tunnemann et al. 2006). Alternatively, the fluorescence detection method used might not be sensitive enough to detect very low concentrations of fluorescent peptide over the background signal.

Next, we wanted to test the intracellular stability of the marker and its localization. This was examined by applying the transducible fluorescent nucleolar marker to living C2C12 myoblasts for 30 min, which as before resulted in nucleolar accumulation, removing the excess extracellular fluorescent peptide by a washing step with PBS and analyzing the cells by confocal microscopy 24 h later. The fluorescent nucleolar label was surprisingly stable showing very bright nucleolar signal 1 day later without detectable decrease in fluorescence intensity or the number of cells marked (Fig. 3c). No proteolytic degradation or intracellular recycling of the FITC-R_{10} was observed within this time period. In addition, the morphology of the cells was not changed, and the cultures grew to confluency indicating no overt effect of the nucleolar marker on cell cycle progression and mitotic division. The structure

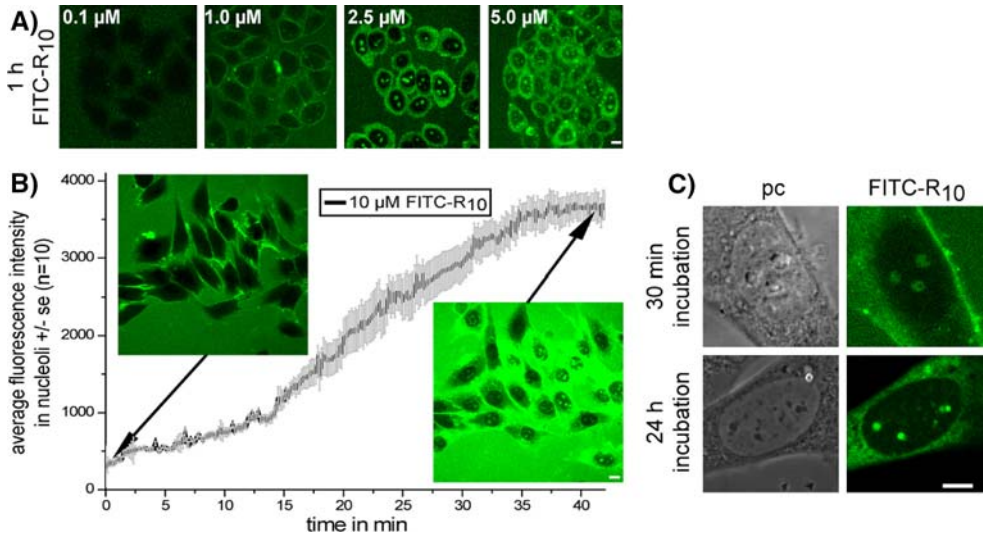

Fig. 3 Kinetics of uptake and intracellular stability of the nucleolar marker The images in **a** show the concentration dependent labeling of the nucleoli in HeLa cells with a threshold at a concentration of 2.5 μM FITC-R_{10}. The kinetics of uptake in **b** display the increase of the fluorescent label in the nucleoli directly after application of 10 μM FITC-R_{10} in medium to living C2C12 cells without a washing step. The uptake started immediately after the application of the nucleolar marker and was clearly visible after 20 min. A maximum of the nucleolar label was reached 40 min after the start of the experiment (see supplementary movie). **c** C2C12 cells were incubated for 30 min with FITC-R_{10} in a medium followed by washing in PBS and imaged 24 h later. Representative images are shown and demonstrate the stability of the transducible nucleolar marker over the 1 day period tested (see also Fig. 2a). Scalebar: 5 μm

A)

	30 min FITC-R10 incubation	24 h FITC-R10 incubation	control (no peptide)
% cells with labeled nucleoli	89%	98%	n.a.
% cells with unlabeled nucleoli	9%	2%	n.a.
% Trypan blue positive cells	2%	< 1 %	2%

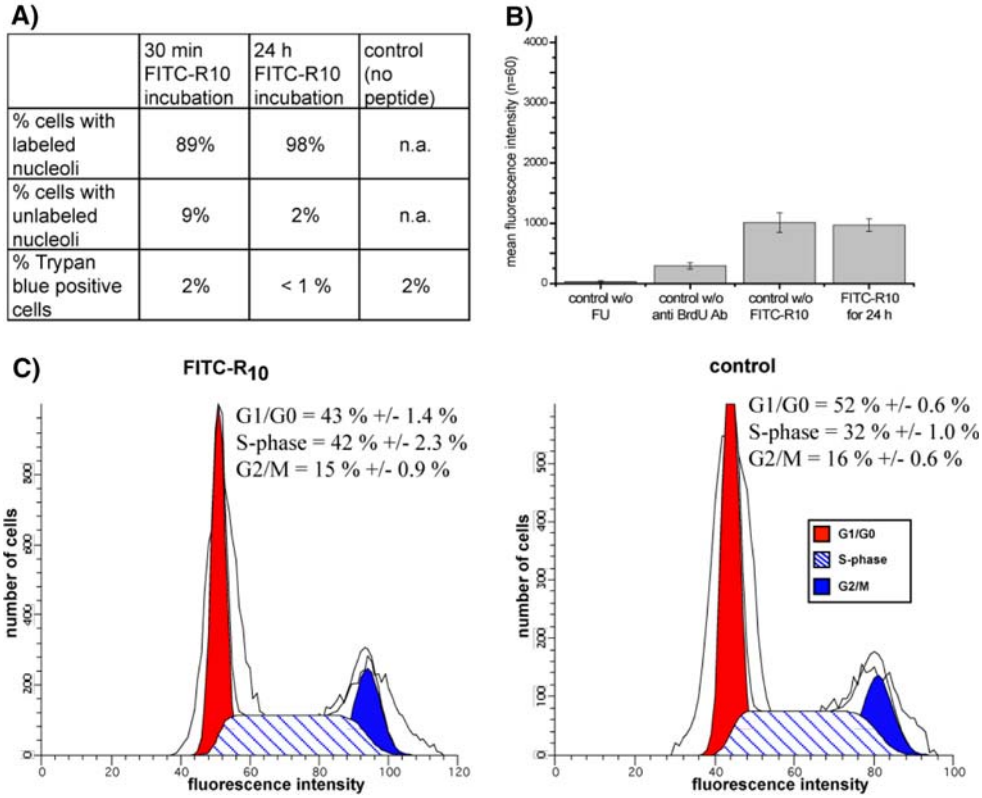

Fig. 4 Effect of the nucleolar marker on the cellular viability, proliferation and rDNA transcription The table in **a** summarizes the percentage of cells with labeled or unlabeled nucleoli and the trypan blue exclusion test after short- and long-term incubation of living cells with 5 μM FITC-R_{10}. In both cases a small number of cells remained unlabeled, whereas the majority of cells showed nucleolar labeling after 30 min and also after 24 h incubation with the nucleolar marker. The quantity of trypan blue positive cells during short- and long-time incubation is comparable to the control cells. The diagram in **b** displays in each bar the mean intensity of fluorescently labeled FU incorporated into rRNA from 60 nuclei measured in confocal images. The signals of labeled rRNA in FITC-R_{10} labeled or unlabeled control cells differed only a little and within the range of the standard deviation. In **c** the histograms represent fitted curves of flow cytometry measurements. HeLa cells were incubated or not with the transducible nucleolar marker for 1 h following a washing step in PBS and staining with PI in the presence of RNase. The percentage of cells in the respective cell cycle stage, and the standard errors were calculated from ten measurements each

and localization of components of the granular component visualized by antibody labeling of B23 was also not altered between untreated cells and samples labeled for 1 and 24 h with FITC-R_{10} (Fig. 2a and data not shown).

To measure the efficiency of nucleolar labeling we determined the percentage of labeled cells at different times after application. Nearly 89% of the cells showed nucleolar fluorescence after 30 min and the number even increased to 98% at 24 h (Fig. 4a). Having FITC-R_{10} in the growth medium for the initial 30 min or during the entire 24 h period yielded no difference in the nucleolar label and the cellular viability (Fig. 4a and data not shown). To further investigate the impact of the peptide transduction on membrane integrity we used the trypan blue exclusion test. No difference was measured between labeled and unlabeled control cells in the number of trypan blue positive cells, which was 1–2% (Fig. 4a).

Next, cells labeled for 24 h with FITC-R_{10} were incubated for 10 min with fluorouridine (FU), which is preferentially incorporated into rRNA, and the incorporated nucleotide was detected by immunostaining to determine the amount of synthesized RNA in comparison to cells without nucleolar marker (Boisvert et al. 2000). The diagram in Fig. 4b displays the mean fluorescence intensity of 60 nuclei measured from low magnification confocal images. FU labeled cells with and without FITC-R_{10} exhibited no difference in the level of incorporated nucleotide, i.e., no effect was detected on rDNA transcription.

Finally, to assess potential effects of FITC-R_{10} on cell proliferation, we subjected HeLa cells with labeled nucleoli and unlabeled control cells to PI staining and flow cytometric analysis of cellular DNA content. The cells were incubated for 1 h with 10 μM FITC-R_{10} and then washed in PBS and grown at 37°C for 24 h. After

this incubation scheme the labeled and untreated control cells were methanol fixed and stained with PI including RNase treatment to measure the DNA amount for cell cycle distribution analysis. The control cells showed a cell cycle profile with 52% of cells in G1/G0, 32% in S-phase and 16% in G2/M. In comparison the cells incubated with 10 μM of the nucleolar marker for 1 h showed some deviations from the control cell cycle profile 24 h post-labeling. The quantity of cells in G1/G0 was decreased to 43%, whereas the cells in S-phase increased to 42% concomitantly. The fraction of G2/M cells remained similar to the control cells (15%; Fig. 4c). These data suggest some delay in cells proceeding through S-phase indicated by an increase and a corresponding decrease of cells in G1/G0. The quantity of cells that exit the S-phase and continued to G2 and the mitotic division was stable also under the influence of the nucleolar marker, which fits the observation of increased confluency in cultures after 24 h.

Altogether, no major effect of the nucleolar marker on either membrane integrity, cell viability and proliferation, RNA synthesis or distribution of nucleolar components was observed at short- or long-incubation times.

Such a label is totally non-invasive and importantly works well in combination with other fluorochromes such as autofluorescent proteins or various dyes. Moreover, most cells not only established cell lines but also non-transfectable primary cultures were transduced with 90% or higher efficiency, and the nucleolar labeling needed only about 20 min to be readily visualized. Curiously, always a few cells remained unlabeled, which could be due to genetic or epigenetic differences in gene expression among the cells in the culture (Tyagi et al. 2001).

We conclude that the FITC labeled deca-arginine peptide does not interfere with major cellular processes at the concentrations tested. The combination of membrane permeability and nucleolar targeting in one peptide makes this transducible fluorescent marker for the nucleoli applicable to most mammalian cells and a novel and non-invasive tool for live cell microscopy.

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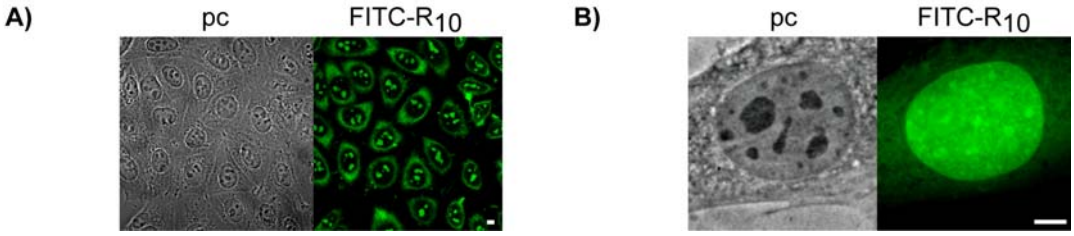
supplementary Fig. 1



Supplementary Figure 1. The nucleolar marker is not removed by fixation. A) HeLa cells were treated with 10 µM FITC-R10 for 60 min then washed and incubated for 1h in growth medium. Finally the cells were fixed with 3.7% formaldehyde in PBS and the sample was mounted on a coverslip for microscopy analysis. The nucleolar label was comparable to the label in living cells and stable during the washing, fixation and mounting treatments. In B) the cell was fixed in 3.7% formaldehyde before the application of FITC-R10 and the fluorescent peptides did not label the nucleoli. Scalebar 5 µm (PDF 52 kb)
Kinetics of FITC-R10 uptake into living C2C12 cells. For description see Fig. 3b